Amendments to the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

1. (currently amended) A method for identifying an immunosuppressive agent comprising:

(a) obtaining at least one population of viable cultured active T cells having intact cell membranes from a cell growth medium under conditions conducive to growth;

(b) combining a first portion of said at least one population with a predetermined amount of at least one test compound dissolved in a solvent for a predetermined period of time at a predetermined temperature thereby generating a first volume;

(c) combining a second portion of said at least one population with an amount of the solvent which was used to dissolve said at least one test compound, for said predetermined period of time at said predetermined temperature thereby generating a second volume;

(d) separately adding to each of said first volume and said second volume a cell permeable reporter compound having at least one measurable property which is responsive to the caspase cascade, wherein said reporter compound comprises

(i) a caspase substrate; and

(ii) a fluorogenic or fluorescent moiety, whereby said at least one measurable property is a change in fluorescence;

(e) measuring said at least one measurable property of said reporter compound in said first volume and thereby measuring the caspase cascade activity of said first volume;

(f) measuring said at least one measurable property of said reporter compound in said second volume and thereby measuring the caspase cascade activity of said second volume;

(g) calculating a first ratio of caspase cascade activity measured for said first volume to said caspase cascade activity measured for said second volume, wherein when the first ratio is greater than one, said at least one test compound kills active T cells and is identified as a potential immunosuppressive agent.

2. (original) The method of claim 1, further comprising:

(a) obtaining at least one population of viable cultured resting T cells having intact cell membranes from a cell growth medium under conditions conducive to growth;

(b) combining said resting T cells with said predetermined amount of said identified immunosuppressive agent dissolved in said solvent for said predetermined period of time at said predetermined temperature thereby generating a third volume;

(c) adding to said third volume said reporter compound having at least one measurable property which is responsive to the caspase cascade;

(d) measuring said at least one measurable property of said reporter compound in said third volume and thereby measuring the caspase cascade activity of said third volume; and,

(e) calculating a second ratio of caspase cascade activity measured for said first volume to said caspase cascade activity measured for said third volume, wherein when the second ratio is greater than one, then said identified immunosuppressive agent is further identified as an active-T-cell-selective immunosuppressive agent.

3. (cancelled)

4. (Original) The method of claim 1 or 2, wherein said at least one test compound is applied to the T cells at a concentration in the range from about 1 picomolar to about 1 millimolar.

5. (original) The method of claim 1 or 2, further comprising adding a permeabilization enhancer in combination with said reporter compound.

6. (original) The method of claims 1 or 2, wherein said predetermined period of time is about 1 minute to about 48 hours; and wherein said predetermined temperature is about 4°C to about 42°C.

7. (original) The method of claim 6, wherein said predetermined period of time is about 24 hours to about 48 hours.

8. (cancelled)

9. (original) The method of claim 1, wherein a plurality of viable cultured active T cell samples are exposed separately to a plurality of test compounds.

10. (original) The method of claim 2, wherein a plurality of viable cultured resting T cell samples are exposed separately to a plurality of test compounds.

11. (original) The method of claims 9 or 10, wherein said plurality of viable cultured cells are in separate wells of a microtiter plate.

12. (original) The method of claim 1, wherein said active T cells are obtained by adding to T cells antibodies to the T cell receptor, Concanavalin A, or Phytohaemagglutinin.

13. (original) The method of claim 1 or 2, wherein said active T cells are obtained from tissue of a patient afflicted with one or more immunopathological symptoms and wherein said resting T cells are from healthy tissue that is not afflicted with the immunopathological symptoms.

14.-27. (cancelled)

28. (currently amended) A method for assaying the potency of a test compound to synergise with a known immunosuppressant by functioning as an activator of the caspase cascade, said method comprising:

(a) obtaining at least one population of viable cultured active T cells having intact cell by culturing T cells in a cell growth medium under conditions conducive to growth and activating the cells;

(b) exposing a first portion of said at least one population to a combination of a predetermined amount of said test compound and a subinducing amount of said known immunosuppressant for a first predetermined period of time, at a first predetermined temperature thereby generating a first volume;

(c) exposing a second portion of said at least one population to an amount of solvent which was used to dissolve the test compound and to said subinducing amount of said known immunosuppressant for said first predetermined period of time at said first predetermined temperature thereby generating a second volume;

(d) adding a cell permeable reporter compound to said first volume and to said second volume, said reporter compound having at least one measurable property which is responsive to the caspase cascade, wherein said reporter compound comprises

(i) a caspase substrate; and

(ii) a fluorogenic or fluorescent moiety, whereby said at least one measurable property is a change in fluorescence;

(e) incubating the resulting mixture of said first volume with said reporter compound for a second predetermined time period at a second predetermined temperature;

(f) incubating the resulting mixture of said second volume with said reporter compound for said second predetermined time period at said second predetermined temperature;

(g) measuring said at least one measurable property of said reporter compound in each of said resulting mixtures and thereby measuring the caspase cascade activity of said first volume and of said second volume; and,

(h) calculating the ratio of measured caspase cascade activities of said first volume to said second volume to determine whether said test compound ~~synergies~~ synergises with said known immunosuppressant as an activator of the caspase cascade.

29. (original) The method of claim 28, wherein a plurality of populations of viable cultured active T cells are exposed separately to a plurality of test compounds.

30. (original) The method of claim 28, wherein said plurality of populations of viable cultured active T cells are in separate wells of a microtiter plate.

31. (currently amended) A method for identifying an immunosuppressive agent comprising:

(a) obtaining viable cultured active T cells having an intact cell membrane;

(b) obtaining viable cultured resting T cells having an intact cell membrane;

(c) separately exposing the active and resting T cells to at least one test compound for a predetermined period of time under predetermined conditions;

(d) adding a cell permeable reporter compound having at least one measurable property which is responsive to the caspase cascade to the active and resting T cells that have been exposed to the at least one test compound, wherein said reporter compound comprises

(i) a caspase substrate; and

(ii) a fluorogenic or fluorescent moiety, whereby said at least one measurable property is a change in fluorescence;

(e) measuring the caspase cascade activity in the active T cells exposed to the at least one test compound by measuring said at least one measurable property; and

(f) measuring the caspase cascade activity in said resting T cells exposed to the at least one test compound by measuring said at least one measurable property, wherein when the caspase cascade activity in the active cells is greater than the caspase cascade activity in the resting cells, the at least one test compound selectively kills active T cells and is an immunosuppressive agent.

32.-35. (cancelled)

36. (new) The method of claim 28 or 31, wherein said at least one test compound is applied to the T cells at a concentration in the range from about 1 picomolar to about 1 millimolar.

37. (new) The method of claim 28 or 31, further comprising adding a permeabilization enhancer in combination with said reporter compound.

38. (new) The method of claims 28 or 31, wherein said predetermined period of time is about 1 minute to about 48 hours; and wherein said predetermined temperature is about 4°C to about 42°C.

39. (new) The method of claim 38, wherein said predetermined period of time is about 24 hours to about 48 hours.

40. (new) The method of claim 31, wherein a plurality of viable cultured active T cell samples are exposed separately to a plurality of test compounds.

41. (new) The method of claim 31, wherein a plurality of viable cultured resting T cell samples are exposed separately to a plurality of test compounds.

42. (new) The method of claims 40 or 41, wherein said plurality of viable cultured cells are in separate wells of a microtiter plate.

43. (new) The method of claim 28 or 31, wherein said active T cells are obtained by adding to T cells antibodies to the T cell receptor, Concanavalin A, or Phytohaemagglutinin.

44. (new) The method of claim 28 or 31, wherein said active T cells are obtained from tissue of a patient afflicted with one or more immunopathological symptoms and wherein said resting T cells are from healthy tissue that is not afflicted with the immunopathological symptoms.